

# Enhanced transcription of c-myc proto-oncogene in spleen lymphocytes from lupus-prone mice during the growing process

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The expression of c-myc proto-oncogene in spleen lymphocytes has been studied in lupus-prone mice (*MRL/Mp-lpr/lpr*), an animal model for the human autoimmune disease systemic lupus erythematosus, during the growing process, in comparison to control mice (*MRL/Mp-+/+*). By Northern blot assay and nuclear run on transcription assay, we demonstrated the enhancement of c-myc proto-oncogene expression in spleen lymphocytes from lupus-prone mice in comparison to control mice and the level of expression of c-myc proto-oncogene increased during the growing process and deterioration of lupus symptoms, such as production of autoantibodies and lymphoproliferation, in this study.

c-myc; Growing process; Lupus-prone mouse

## 1. INTRODUCTION

Systemic autoimmune disorders, such as systemic lupus erythematosus, are characterized by immunological dysfunction and connective tissue inflammation. William et al. [1] claimed that most autoimmune disorders could be considered benign proliferate disorders. Most of the fundamental causes of autoimmune disorders remain obscure. Proto-oncogenes, including c-myc, are expressed in normal lymphocytes after stimulation with appropriate mitogens [2,3], and may play key roles in growth and differentiation. *MRL/Mp-lpr/lpr* mice, an animal model of human systemic lupus erythematosus, develop age-dependent progressive lupus symptoms, such as production of high titer autoantibodies and accumulation of abnormal subset of lymphocytes [4].

In the present study, we examined the expression of c-myc proto-oncogene in spleen lymphocytes from lupus-prone mice (*MRL/Mp-lpr/lpr*) during the growing process in comparison to control mice (*MRL/Mp-+/+*). We also tried to examine the relationships between the elevated level of c-myc transcription and deterioration of autoimmune symptoms. The results indicate a good correlation between these parameters and their possible significance in the pathophysiology of lupus is discussed.

## 2. MATERIALS AND METHODS

### 2.1. Animal

*MRL/Mp-lpr/lpr* (*MRL/lpr*) and *MRL/Mp-+/+* (*MRL/+*) mice were obtained from Jackson Laboratory (Bar Harbor, ME) and maintained in our laboratory. We carefully checked for lupus symptoms, such as production of autoantibodies, proteinuria due to renal disorder and lymphadenopathy during the growing process in both strains [4].

### 2.2. Preparation of spleen lymphocytes

Spleen lymphocytes from lupus-prone mice (*MRL/lpr*) and control mice (*MRL/+*) were isolated by gentle separation on a wire mesh. The erythrocytes were removed by Ficoll-Paque, pH 7.2 (Pharmacia Fine Chemicals, NJ). The cell population was over 97% viable (Trypan blue exclusion).

### 2.3. Preparation of RNA and Northern blot assay

The isolation of polyadenylated RNA from spleen lymphocytes was done as described by Swab et al. [5]. RNA was quantitated spectrophotometrically at 260 nm and applied on 0.8% formaldehyde-agarose gels. Samples were transferred to a nylon membrane (Pall, NY), and baked under vacuum at 80°C for 2 h. Filters were hybridized overnight at 42°C with <sup>32</sup>P-labelled probes for c-myc (Oncor Inc., CA) or actin (Wako Pure Chemical Industries, Ltd, Japan). After washing in 1 × SSC (150 mM NaCl, 15 mM sodium citrate), 0.1% SDS at 60°C, filters were exposed to Kodak AR X-Omat films at -70°C.

### 2.4. Nuclear run on transcription assay

Nuclei were prepared from the cells by lysing the cells in the solution which contained 10 mM Tris (pH 7.5), 2 mM MgCl<sub>2</sub>, 3 mM CaCl<sub>2</sub>, 5 mM DTT and 0.02% of NP40, with subsequent centrifugation through 2 M sucrose solution. Three million nuclei were suspended into 100 µl of 50% glycerol solution with 50 mM Tris (pH 7.5), 5 mM MgCl<sub>2</sub> and 0.1 mM EDTA. The suspension of nuclei was

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immediately mixed with an equal volume of buffer containing 0.2 M KCl, 5 mM MgCl<sub>2</sub>, 5 mM DTT, 1 mM of ATP, CTP, GTP, and 200 units of RNasin (ribonuclease inhibitor, 500 units, Amersham International plc, England). The preparation was then incubated at 28°C for 20 min after addition with 50  $\mu$ Ci of <sup>32</sup>P-radiolabelled UTP (3000 mCi/ml, Amersham Inc.). To the preparation were added SDS and EDTA solution to a final concentration of 1% and 5 mM respectively, followed by treatment with proteinase K (1 mg/ml) at 42°C for 30 min. RNA was extracted with phenol and chloroform from the preparation and precipitated with ethanol. The pellet was resuspended into 3 ml of hybridization buffer which contained 50% of formamide, 0.75 M NaCl, 0.5% of SDS, 2 mM of EDTA, 50 mM of Hepes (pH 7.0), one tenth dilution of Denhardt's solution and denatured salmon sperm DNA (500  $\mu$ g/ml) [6]. Finally, the preparation was applied to the nitrocellulose filter onto which the *c-myc* probe (Oncor Inc. USA) or  $\beta$ -actin probe (Wako Pure Chemical Industries, Ltd, Japan) had been dotted. After 24 h incubation, the filter was washed three times in 0.2  $\times$  SSC and 0.1% of SDS at 45°C, dried and exposed to X-ray film with intensifying screen at -70°C. In some experiments, the hybridized dot was excised from the filter and directly counted with a  $\beta$ -counter [7].

### 3. RESULTS AND DISCUSSION

As shown in fig.1, we found the enhancement of RNA expression of *c-myc* proto-oncogene in spleen lymphocytes from lupus-prone mice (*MRL/lpr*), as compared with healthy control mice (*MRL/+*). We detected no significant change of the RNA expression of actin gene between them (data not shown). We examined the transcriptional level of *c-myc* proto-oncogene in spleen lymphocytes from lupus-prone and control mice by nuclear run on transcription assay. We show the representative data of nuclear run on assay in fig.2. As shown in fig.2, we first demonstrated that the enhancement of transcriptional level of *c-myc* proto-oncogene was important for the increased RNA for *c-myc* proto-oncogene in spleen lymphocytes from lupus-

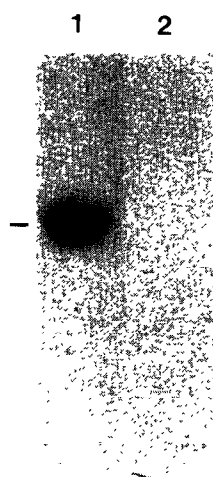


Fig.1. Northern blot assay of *c-myc* proto-oncogene in spleen lymphocytes from lupus-prone and control mice. 1, *MRL/Mp-lpr/lpr* (2 months after birth); 2, *MRL/Mp-+/+* (2 months after birth).

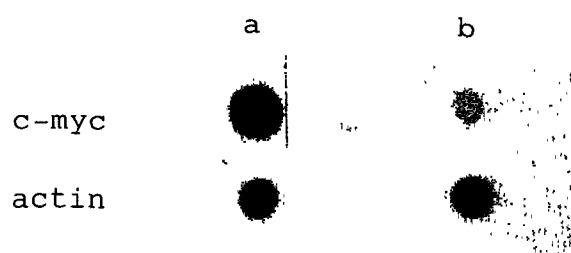


Fig.2. Nuclear run on transcription assay of *c-myc* proto-oncogene and actin gene in spleen lymphocytes from lupus-prone and control mice. a, *MRL/Mp-lpr/lpr* (2 months after birth); b, *MRL/Mp-+/+* (2 months after birth).

prone mice. We found no significant change of transcription of actin gene between lupus-prone and control mice. This amplification was unlikely to be due to gross genetic changes because when the DNA was digested with *Eco*RI and *Hind*III, and examined by Southern blot analysis with *c-myc* specific probe, no significant gene amplifications or gross translocations were observed (data not shown). The *c-myc* gene product has DNA-binding ability [8], localized in the nucleus [9] and is induced in proliferating or differentiating cells [10]. Deregulation of *c-myc* transcription or translocation may disrupt the normal cell control. Since the enhancement of transcription of *c-myc* proto-oncogene was detected in spleen lymphocytes from lupus-prone mice, there are many activational stages and/or steps of spleen lymphocytes from lupus-prone mice.

We further examined the transcriptional change of *c-myc* proto-oncogene during the growing process in lupus-prone mice (fig.3). As shown in fig.3, we found

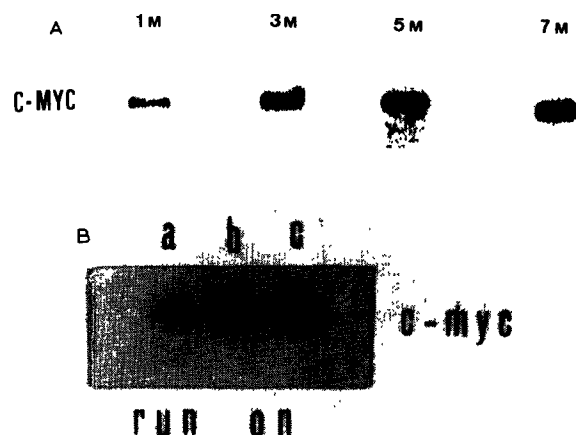


Fig.3. Expression of *c-myc* proto-oncogene in spleen lymphocytes from lupus-prone mice during the growing process. (A) Northern blot assay of *c-myc* RNA in spleen lymphocytes from *MRL/Mp-lpr/lpr* mice (1, 3, 5 and 7 months after birth). (B) Nuclear run on transcription assay of *c-myc* proto-oncogene expression in spleen lymphocytes from *MRL/Mp-lpr/lpr* mice. a, 1 month after birth; b, 3 months after birth; c, 5 months after birth.

Table 1

Correlation between *c-myc* expression in spleen lymphocytes and lupus symptoms in lupus-prone mice (*MRL/Mp-lpr/lpr*)

Period after birth	<i>c-myc</i> expression	Proteinuria level	Production of autoantibodies	Lymphadenopathy level
2 days	+	—	—	—
2 weeks	+	—	—	—
2 months	++	+	+	+
4 months	+++	++	++	++
6 months	++++	+++	+++	+++

Expression of *c-myc* was evaluated by Northern blot assay and nuclear run on assay. Levels of proteinuria, production of serum autoantibodies and lymphadenopathy were evaluated by the usual laboratory and pathological examinations [4]

the increase of expression of *c-myc* proto-oncogene in spleen lymphocytes from lupus-prone mice during the growing process. In table 1, which summarizes the results, we also found a positive correlation between the transcriptional enhancement of *c-myc* proto-oncogene and deterioration of lupus symptoms, such as production of autoantibodies, proteinuria due to lupus-related renal disorders and lymphadenopathy [4]. This result could suggest that the expression of *c-myc* proto-oncogene in spleen lymphocytes from lupus-prone mice could correlate with the disease activity of lupus. With respect to the pathophysiological meaning of lupus for the enhancement of *c-myc* gene transcription in spleen lymphocytes from lupus-prone mice, we found that the enhancement of the *c-myc* gene transcription preceded the exacerbation of lupus symptoms and changes in laboratory data such as the level of proteinuria and serum autoantibodies. Thus, we expect that the examination of the mechanisms and factors which affect the enhanced transcription of *c-myc* proto-oncogene can give us the essential clue to the evaluation of the pathophysiology of lupus in lupus-prone mice.

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